

# Fingernails as biological indices of metal exposure

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Metal determination in human tissues is the most common application of biological monitoring for screening, diagnosis and assessment of metal exposures and their risks. Various biopsy-materials may be used. This paper deals with the quantitative determination of Cd, Pb, Cr, Mn, Fe, Ni, Cu, and Zn concentrations in nails of male subjects exposed to these metals alongwith their respective controls, while working in locomotive, carriage and roadways workshops, and lead battery factories. The levels of Cd, Pb, Cr, Mn, Fe, Ni, Cu and Zn in fingernails, assayed by atomic absorption spectrophotometry, were compared with their respective controls by student 't' test. All the obtained values were correlated to the personal and medical history of the subjects under study. Significantly high levels of Cd, Pb, Cr, Fe, Ni, Cu and Zn were present in smokers, compared to nonsmokers. The concentrations of Cd, Pb, Cr, Mn and Fe were not significantly high in vegetarian subjects. It was also observed that there is no contribution of liquor towards nail-metal concentration. Significant correlations were observed between skin disease and Cr, Mn, Fe, Cu; hypertension and Cd, Mn, Cu; mental stress and Cd, Pb, Mn, Ni, Cu, Zn; diabetes and Cr, Mn, Ni; chest pain and Pb; respiratory trouble and Cr, Mn, Fe, Ni, Zn; tuberculosis and Zn; acidity and Cd; and ophthalmic problems and Mn, Fe, Ni, and Zn.

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## 1. Introduction

Industrialization, urbanization, mining operations, increased vehicular traffic and use of fertilizers and pesticides in agriculture have resulted in increased metal contamination in our environment. Not only the occupationally exposed workers (high-risk population group) but the community at large (low-risk population group) may suffer due to increased metal pollutants in the environment. Though certain essential trace elements are required in trace amounts for various physiological processes; but, at higher concentrations, these micronutrients tend to be toxic and derange various physiological processes, leading thereby to diseases. Therefore, it is important to determine the metal concentrations in humans to monitor and assess their impact on human health (Florence 1990; Oluwole *et al* 1994; Ather and Vohora 1995; Satake *et al* 1997; Nath 2000). Among various biopsy materials; blood, hair, nail, teeth and other body fluids may be used as bioindicators

for this purpose. Unlike blood that gives transient concentrations, nails can provide a continuous record of trace element concentrations of the body (Wilhelm and Hafner 1991). They can be easily sampled and analysed for accumulated toxic and essential metals in the tissue. Studies on nails as bioindicators have been reported by Vance *et al* (1988), Hayashi *et al* (1993), Oluwole *et al* (1994) and Chaudhary *et al* (1995). However, studies on correlation of nail-metal levels with different parameters, as well as with various health disorders are scarce.

As a continuation of our earlier studies (Mehra and Juneja 2003a,b,c, 2004), here we report the nail-trace metal levels (Cd, Pb, Cr, Mn, Fe, Ni, Cu and Zn) in different age groups of subjects with varying personal habits and prone to the hazards of trace metals in their occupational environment. For this study, we have used fingernails as biopsy material. Male subjects of matching ages in different groups were taken as controls who were selectively chosen on basis of their specific habits so as to

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avoid influence of one parameter on the other. For this, the subjects were personally interviewed and a questionnaire with details of their personal and medical history was filled.

## 2. Materials and methods

### 2.1 Sample collection

For collection of nail samples, volunteers were asked to wash their hands thoroughly with distilled water and medicated soap devoid of metal contamination, followed by drying with clean towel or tissue paper to remove any external contamination. Using clean stainless steel scissors fingernail samples of male subjects (age 11–60 years) were collected from the fingers. For subsequent analysis, each of the nail sample (1 g) was sealed in plastic cover till it was washed, dried, digested and converted into a water-clear solution.

### 2.2 Procurement of requisite details of subjects

The personal and medical history, alongwith relevant details of the subjects taken for study were obtained through a questionnaire based on recommendation of World Health Organisation (WHO). The information required to be filled in the proforma included sex, age, personal habits (smoking, drinking and food), place of residence, occupation and any possible prior metal exposure.

### 2.3 Washing

For washing of nails, the nail samples were scrapped and cleaned of dust particles with nonionic detergent (Triton X-100) following a standardized washing procedure (Gammelgaard *et al* 1991). Subsequently the nails were soaked in acetone to remove external contamination, rinsed five times with deionized water, dried in an oven and stored in a dessicator.

### 2.4 Wet-acid digestion and preparation of water-clear solution

For wet acid digestion, the dried nail samples were digested with 10 ml of 6 : 1 mixture of concentrated nitric and perchloric acid kept overnight at room temperature to prevent excessive foaming and subsequently heated at 160–180°C until the mixture was water clear and less than 1 ml of the solution remained. (The organic matter is destroyed by wet-acid digestion and the solution containing the metal in its elemental form is obtained.) Each sample solution was then diluted with 0.1 N nitric acid.

### 2.5 Analysis

The concentrations of the metals were assayed by using Perkin Elmer AAS model 250 Plus with air acetylene flame. A series of standards were prepared in deionized water for instrumental calibration by diluting commercial standards containing 1000 ppm of the metals. All reagents used were of analytical grade procured from Merck (Germany) free from any metal contamination. A number of blanks were prepared for minimization of contaminated errors. The main instrumental parameters (like band width, lamp current and wavelength) for estimation of metals by atomic absorption spectrophotometer were set-up separately for each metal. Adequate quality control was ensured by way of interlaboratory analysis of representative samples at Industrial Toxicological Research Centre (Lucknow), National Physical Laboratory (New Delhi), National Institute of Occupational Health (Ahmedabad) and Indian Bureau of Mines (Ajmer) observing the standard protocol in measurements. The results obtained were in agreement with one another. In view of nonavailability of nail Certified Reference Material, this study is a step towards preparation of standard reference material for laboratory use.

### 2.6 Statistical analysis

The values of metal levels in nails are presented as arithmetic mean in µg/g with standard deviation and tabulated to illustrate concentration profile over each group. The statistical significance of mean values between different groups has been determined by applying student 't' test. The level of significance was set at  $P < 0.05$ .

## 3. Results and discussion

The results of the quantitative analyses of fingernails for Cd, Pb, Cr, Mn, Fe, Ni, Cu and Zn are given in table 1. The samples analysed were categorized according to their personal habits (smoking, food and drinking) and medical history. The pathological symptoms related to different health disorders in subjects working in metal-polluted environment are skin disease, hypotension, hypertension, mental stress, diabetes, chest pain, respiratory trouble, cardiovascular problem, tuberculosis, acidity, ophthalmic problem and hepatitis B. Age- and sex-matched controls were also selected from the same work environment i.e. similar sampling sites. The values of almost all metals investigated except Mn were significant, Cd and Cr being particularly higher. Smoking was found to be a contributing factor to higher bioaccumulation of Cd as also reported by other researchers (Chattopadhyay *et al* 1990; Sukumar and Subramanian 1992). The high Cr concentra-

tion obtained may be attributed to the Cr content in tobacco leaves which might get incorporated in the leaves from the soil. High level of Cr in smokers have also been reported by Sukumar and Subramanian (1992). Besides tobacco, main sources of metal in cigarettes are wrapping paper and the filter. However, effect of smoke expired by smokers on nonsmokers on inhalation, was eliminated by choosing subjects who do not remain in the vicinity of

smokers by and large. High level of iron observed is probably due to the presence of iron oxide fumes in the environment of workplace as various processes involved emanate oxides of iron. Significant Ni levels in smokers compared to nonsmokers have also been reported by Wolfspurger *et al* (1994) at significant level of  $P < 0.005$ . Besides this, the inhalation of vapors of nickes carbonyl from the worktype (welding, fitting, etc.) also cause ele-

**Table 1.** Mean ( $\mu\text{g/g} \pm \text{SD}$ ) and statistical significance of trace metals in nails with respect to various parameters.

Subjects	No. of samples	Age in years	Cd	Pb	Cr	Mn	Fe	Ni	Cu	Zn
With respect to smoking habit										
Smokers	—	11–20	—	—	—	—	—	—	—	—
Non-smokers	20	11–20	$0.87 \pm 0.61$	$16.37 \pm 6.83$	$87.06 \pm 26.32$	$17.75 \pm 9.92$	$288.65 \pm 40.96$	$56.36 \pm 13.19$	$8.14 \pm 4.75$	$110.29 \pm 4.38$
Smokers	20	21–30	$0.76 \pm 0.81^*$	$56.68 \pm 19.27^*$	$103.60 \pm 39.73$	$11.71 \pm 5.29$	$424.68 \pm 79.43^*$	$68.29 \pm 17.81$	$4.13 \pm 1.42^*$	$161.48 \pm 33.43$
Non-smokers	20	21–30	$0.42 \pm 0.12$	$37.50 \pm 7.57$	$102.64 \pm 16.52$	$20.04 \pm 7.18^*$	$156.98 \pm 28.37$	$63.26 \pm 15.36$	$1.58 \pm 0.76$	$184.44 \pm 32.42^*$
Smokers	20	31–40	$3.56 \pm 1.81^*$	$76.09 \pm 28.13^*$	$126.93 \pm 52.59$	$16.94 \pm 1.71$	$326.50 \pm 53.42^*$	$78.17 \pm 15.65^*$	$6.07 \pm 1.13^*$	$192.88 \pm 42.61$
Non-smokers	20	31–40	$1.11 \pm 0.83$	$49.86 \pm 17.76$	$131.13 \pm 50.40$	$22.39 \pm 8.81^*$	$259.87 \pm 46.49$	$65.26 \pm 13.98$	$4.31 \pm 1.58$	$190.29 \pm 34.26$
Smokers	20	41–50	$1.19 \pm 0.72^*$	$80.88 \pm 39.94^*$	$129.67 \pm 44.01$	$13.87 \pm 1.39$	$382.95 \pm 102.09^*$	$70.91 \pm 17.06$	$6.34 \pm 1.32^*$	$166.36 \pm 29.78^*$
Non-smokers	20	41–50	$0.73 \pm 0.31$	$60.74 \pm 42.91$	$128.47 \pm 39.59$	$13.01 \pm 4.92$	$288.58 \pm 57.52$	$66.89 \pm 29.17$	$3.07 \pm 1.39$	$132.21 \pm 32.15$
Smokers	20	51–60	$1.27 \pm 0.59^*$	$92.27 \pm 53.57^*$	$156.18 \pm 29.13^*$	$15.86 \pm 5.97$	$316.20 \pm 94.27^*$	$93.31 \pm 26.19^*$	$26.07 \pm 16.34^*$	$172.33 \pm 36.85$
Non-smokers	20	51–60	$0.69 \pm 0.31$	$20.02 \pm 15.78$	$92.64 \pm 26.62$	$18.87 \pm 8.78$	$273.75 \pm 78.15$	$61.59 \pm 12.86$	$10.17 \pm 5.82$	$160.73 \pm 32.72$
With respect to food habit										
Vegetarian	13	11–20	$0.80 \pm 0.21$	$16.45 \pm 4.26$	$68.45 \pm 15.54$	$26.58 \pm 6.67$	$327.30 \pm 94.23$	$62.35 \pm 16.12$	$9.45 \pm 1.92^*$	$177.95 \pm 15.42^*$
Non-vegetarian	8	11–20	$0.99 \pm 0.72$	$15.02 \pm 3.57$	$89.81 \pm 16.52^*$	$39.71 \pm 16.17^*$	$358.55 \pm 121.59$	$74.73 \pm 24.17$	$7.14 \pm 3.56$	$162.26 \pm 25.64$
Vegetarian	14	21–30	$0.41 \pm 0.23$	$19.84 \pm 5.84$	$98.27 \pm 21.84$	$20.01 \pm 9.43$	$340.21 \pm 118.79$	$42.83 \pm 14.58$	$8.96 \pm 1.35^*$	$182.28 \pm 22.60^*$
Non-vegetarian	15	21–30	$1.01 \pm 0.43^*$	$33.46 \pm 7.92^*$	$102.79 \pm 30.29$	$43.19 \pm 10.17^*$	$503.86 \pm 246.33^*$	$62.01 \pm 17.47^*$	$6.32 \pm 1.51$	$152.14 \pm 23.23$
Vegetarian	16	31–40	$0.73 \pm 0.61$	$15.65 \pm 2.14$	$112.24 \pm 37.85$	$19.62 \pm 6.49$	$374.13 \pm 109.35^*$	$76.77 \pm 13.82$	$14.36 \pm 3.50^*$	$210.33 \pm 36.41$
Non-vegetarian	33	31–40	$1.31 \pm 0.71^*$	$50.63 \pm 13.70^*$	$135.95 \pm 42.45^*$	$29.43 \pm 10.59$	$298.32 \pm 112.21$	$84.57 \pm 26.78$	$9.11 \pm 4.20$	$197.36 \pm 21.29$
Vegetarian	17	41–50	$1.88 \pm 0.43$	$23.70 \pm 7.81$	$85.91 \pm 36.07$	$14.81 \pm 6.64$	$314.59 \pm 122.20$	$85.48 \pm 19.87$	$6.89 \pm 3.10$	$181.22 \pm 22.79$
Non-vegetarian	54	41–50	$1.23 \pm 0.42$	$36.66 \pm 10.63^*$	$93.14 \pm 8.32$	$16.46 \pm 3.96$	$329.27 \pm 108.07^*$	$79.07 \pm 24.76$	$7.82 \pm 2.39$	$170.15 \pm 39.81$
Vegetarian	13	51–60	$0.82 \pm 0.56$	$22.41 \pm 8.83$	$129.52 \pm 43.96$	$18.67 \pm 6.29$	$311.14 \pm 90.97$	$89.10 \pm 26.75$	$11.31 \pm 2.96^*$	$169.17 \pm 18.68$
Non-vegetarian	19	51–60	$1.03 \pm 0.72$	$35.23 \pm 15.18^*$	$116.24 \pm 49.82$	$17.90 \pm 7.41$	$356.68 \pm 91.42$	$84.95 \pm 29.83$	$6.14 \pm 2.49$	$174.33 \pm 29.86$
With respect to drinking habit										
Liquorusers	—	11–20	—	—	—	—	—	—	—	—
Liquorusers	20	11–20	$0.87 \pm 0.61$	$18.61 \pm 11.48$	$72.52 \pm 18.09$	$17.33 \pm 4.03$	$288.65 \pm 40.95$	$63.23 \pm 9.06$	$8.48 \pm 1.55$	$110.29 \pm 4.38$
Liquorusers	—	21–30	—	—	—	—	—	—	—	—
Liquorusers	24	21–30	$0.59 \pm 0.34$	$19.18 \pm 8.21$	$78.92 \pm 16.66$	$18.35 \pm 5.10$	$354.36 \pm 86.92$	$48.26 \pm 16.17$	$9.35 \pm 0.94$	$147.38 \pm 26.82$
Liquorusers	32	31–40	$2.01 \pm 1.11^*$	$33.65 \pm 9.52$	$86.29 \pm 35.12$	$19.23 \pm 6.37$	$312.81 \pm 91.07$	$61.51 \pm 14.44$	$10.18 \pm 2.46$	$186.72 \pm 23.15$
Liquorusers	36	31–40	$0.89 \pm 0.42$	$30.61 \pm 14.03$	$99.88 \pm 32.13$	$17.70 \pm 4.67$	$364.13 \pm 98.20^*$	$66.63 \pm 23.64$	$12.25 \pm 1.86^*$	$207.66 \pm 35.92^*$
Liquorusers	34	41–50	$0.91 \pm 0.79$	$19.75 \pm 5.83$	$84.44 \pm 7.45$	$14.40 \pm 4.04$	$296.98 \pm 82.65$	$53.33 \pm 18.05$	$7.16 \pm 1.13$	$167.82 \pm 19.62$
Liquorusers	60	41–50	$0.72 \pm 0.42$	$23.51 \pm 10.36$	$102.74 \pm 30.43$	$13.37 \pm 2.26$	$400.61 \pm 15.43^*$	$7.86 \pm 28.91^*$	$8.16 \pm 3.15$	$172.27 \pm 24.75$
Liquorusers	12	51–60	$1.13 \pm 0.71$	$30.29 \pm 8.53^*$	$83.96 \pm 18.75$	$18.15 \pm 4.32$	$321.23 \pm 107.31^*$	$81.32 \pm 36.83$	$6.89 \pm 1.44$	$159.04 \pm 13.47$
Liquorusers	19	51–60	$0.91 \pm 0.39$	$24.51 \pm 11.18$	$101.95 \pm 36.15$	$16.29 \pm 5.34$	$255.43 \pm 95.53$	$73.36 \pm 28.18$	$9.34 \pm 3.38^*$	$158.77 \pm 14.68$
With respect to health disorders										
Controls	20	—	$0.99 \pm 0.32$	$20.21 \pm 6.17$	$86.62 \pm 33.80$	$10.17 \pm 2.09$	$286.61 \pm 55.61$	$56.24 \pm 13.48$	$7.63 \pm 1.46$	$180.60 \pm 45.34$
Skin Disease	20	—	$1.08 \pm 0.53$	$20.00 \pm 7.12$	$168.55 \pm 49.52^*$	$25.65 \pm 10.02^*$	$368.25 \pm 77.25^*$	$58.04 \pm 26.28$	$12.43 \pm 3.94^*$	$184.03 \pm 37.69$
Hypotension	20	—	$1.02 \pm 0.78$	$20.03 \pm 3.81$	$90.34 \pm 19.86$	$11.94 \pm 5.91$	$303.31 \pm 62.18$	$55.86 \pm 24.27$	$10.72 \pm 8.69$	$200.55 \pm 97.64$
Hypertension	20	—	$1.31 \pm 0.51^*$	$16.25 \pm 6.53$	$80.14 \pm 16.64$	$17.47 \pm 4.30^*$	$308.13 \pm 59.94$	$38.49 \pm 10.44$	$16.94 \pm 7.06^*$	$153.12 \pm 22.92$
Mental stress	20	—	$2.01 \pm 1.19^*$	$38.92 \pm 8.82^*$	$100.54 \pm 48.70$	$16.85 \pm 10.96^*$	$306.79 \pm 48.86$	$82.57 \pm 23.66^*$	$18.97 \pm 6.33^*$	$286.59 \pm 40.03^*$
Diabetes	20	—	$0.98 \pm 0.52$	$19.23 \pm 4.16$	$170.84 \pm 56.48^*$	$25.64 \pm 5.97^*$	$294.70 \pm 86.78^*$	$104.93 \pm 35.15^*$	$7.82 \pm 1.69$	$178.27 \pm 22.78^*$
Chest pain	20	—	$1.03 \pm 0.58$	$129.42 \pm 61.17^*$	$83.30 \pm 11.32$	$8.78 \pm 2.39$	$273.72 \pm 48.75$	$49.43 \pm 18.84$	$8.01 \pm 2.07$	$156.49 \pm 27.34$
Respiratory trouble	20	—	$1.02 \pm 0.71$	$15.92 \pm 5.32^*$	$172.92 \pm 66.64^*$	$32.65 \pm 12.57^*$	$574.93 \pm 87.79^*$	$121.01 \pm 28.64^*$	$7.75 \pm 1.49$	$242.97 \pm 23.45^*$
Tuberculosis	20	—	$0.81 \pm 0.52$	$24.01 \pm 7.12$	$79.22 \pm 24.85$	$11.74 \pm 7.18$	$297.56 \pm 83.22$	$48.22 \pm 9.26$	$6.82 \pm 2.91$	$253.94 \pm 32.11^*$
Acidity	20	—	$5.63 \pm 4.59^*$	$13.15 \pm 2.31$	$81.31 \pm 28.59$	$11.50 \pm 5.17$	$226.98 \pm 98.47$	$26.28 \pm 19.52$	$6.48 \pm 1.81$	$150.12 \pm 14.29$
Ophthalmic disease	20	—	$0.71 \pm 0.43$	$14.16 \pm 4.69$	$74.32 \pm 30.61$	$16.78 \pm 6.49^*$	$598.29 \pm 146.17^*$	$98.23 \pm 46.26^*$	$7.86 \pm 1.56$	$233.34 \pm 54.28^*$
Hepatitis b	20	—	$1.02 \pm 0.88$	$8.64 \pm 3.16$	$84.19 \pm 28.49$	$8.81 \pm 3.36$	$211.53 \pm 38.41$	$56.05 \pm 22.50$	$6.18 \pm 1.73$	$125.23 \pm 25.39$

\*Values significant at  $P < 0.05$  with respect to their respective controls.

vation in Ni levels. The possible source of Cu in smokers may be due to insecticidal sprays on soil-producing tobacco.

Student 't' test between vegetarian and non-vegetarian subjects reveal that Cu and Zn was significant in vegetarian subjects in contrast to Cd, Pb, Cr, Mn and Fe. Chmielnicka and Cherian (1986) have reported that beef, and meat contains highest amount of Cd compared to vegetarian food. Significantly high levels of Mn were found in nails of nonvegetarian subjects in lower-age groups. It has been reported that dietary fibre has greatest negative effect on Mn bioavailability. From the table it is clear that Fe, a nutritionally essential metal, was found to be significant in nails of nonvegetarian subjects particularly between 21 and 30 years age group and in vegetarian subjects between 31 and 40 years. Such results may be possible because nonvegetarians may also consume vegetarian diet.

Parametric test between liquor users and liquor nonusers reveal that liquor is not a contributing factor to metal accumulation, since apparently there is no source of metals during liquor production and therefore, as such no correlation exists (Pandey 1994). Although data in relation to this parameter is scarce, high levels of Cu in liquor nonusers could be due to exposure.

Significant test between controls and subjects showing pathological symptoms of various diseases reveal significant correlations between skin disease and Cr, Mn, Fe, Cu; hypertension and Cd, Mn, Cu; mental stress and Cd, Pb, Mn, Ni, Cu, Zn; diabetes and Cr, Mn, Ni; chest pain and Pb; respiratory trouble and Cr, Mn, Fe, Ni, Zn; tuberculosis and Zn; acidity and Cd; and ophthalmic trouble and Mn, Fe, Ni, Zn. The positive correlations between skin disease and Cr, Mn, Fe and Cu reveal that these metals play some role in causing skin disorders. It has been reported in literature that Cr in higher concentration is associated with skin lesions (Mido and Satake 1995) and Ni with skin allergies (Peters *et al* 1991; and Menne *et al* 1987). Cd, Mn and Cu have been observed to be causative factor for hypertensive subjects. Our results for Cd are also supported by those of other workers. Significant levels have been obtained from male hypertensives who were not occupationally exposed subjects, but suffering from hypertension (Sukumar and Subramanian 1992).

Significant relation was observed between nail Cd, Pb, Mn, Ni, Cu, Zn and mentally stress subjects. Clinically high lead encephalopathy in children with high exposure to lead (Nath 2000), neurological complaint similar to Parkinson's disease due to Mn (Bell 1988) has been reported. The neurological disorder known as manganism may result from occupational exposure to manganese dust and fumes (Krishana Murti and Vishwanathan 1991). Workers with chronic headache and dizziness have higher levels of Cr, Mn and Pb in the hair, working in fireworks factory (Sukumar and Subramanian 1992).

Lead was found to be significant in subjects with complaint of chest pain showing a relationship between lead levels in exposed subjects and heart ailments. Lead has been reported to cause heart ailments by raising the blood pressure through its attack on the specific sites and cellular elements of the nervous system (Seth 1998).

In our study, Cr, Mn, Fe, Ni and Zn levels in workers suffering from respiratory trouble were significantly high compared to controls, whereas Cd, Pb and Cu were not significant. However, this observation cannot be solely attributed to the disease factor. The significant levels of Cr, Mn and Ni in diabetic patients; Zn in tuberculosis patients; Cd in acidity cases; Mn, Fe, Ni and Zn in subjects with ophthalmic problems and no metal of significance in hepatitis B subjects found in our study, need further investigation.

Trace/toxic elements play a vital role in human health, yet there is obviously a great deal of concern about the optimum intake and the safe range of each element. More investigations are required. Significant levels of these metals in nail samples indicate the presence of these metals in the environment in the workplace of the subjects, as well as their proneness to the illness and hazards of these metals, in cases of long term exposure.

An overall view of the results obtained for trace/toxic metals and its corelationship with different diseases although establish the use of nails as a diagnostic tool but still more number of studies on a larger portion of the population will enhance the merit of such investigations and their applications in medical and forensic sciences. They will lead to preparation of standard reference material of nail by adopting necessary quality control measures and enhance the reliability of the results. Interlaboratory analysis is therefore being done to help establish the use of nails as bioindicator for metals. It is also deemed essential that certain preventive measures including use of hand gloves and masks should be taken to safe guard the health of the people exposed to metals.

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